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Erucylphosphohomocholine-induced apoptosis in human glioma cells: role of the oligomycin-sensitive F0 part of mitochondrial H+-ATP-synthase

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Erucylphosphohomocholine (ErPC3), representative of a new class of antitumor agents, which target biomembranes and induce cancer cell apoptosis, has been shown to be a promising preclinical anticancer agent. However, the precise mechanisms by which ErPC3 displays its antitumor activities are unknown. In previous studies we demonstrated that ErPC3 activates the mitochondrial apoptotic pathway via the 18 kDa Translocator Protein (TSPO), which in turn causes the MPTP to open leading to collapse of the mitochondrial membrane potential, the first stage of the mitochondrial apoptosis cascade. Cyclosporin A and oligomycin protected glioma cell lines against ErPC3-induced apoptosis.

In this study, we investigated in more detail the significance of the membraneous F0 component of H^+-ATP-synthase in ErPC3-induced PTP opening and apoptosis with the aid of different inhibitors, e.g. oligomycin, applied to human glioblastoma cells, U87MG and U118MG. Furthermore, we measured cytochrome c release, apoptosis, and cellular ATP levels in this paradigm.

In these cells ErPC3-induced apoptosis was insensitive to effects of inhibitors of the mitochondrial respiratory chain and uncouplers of oxidative phosphorylation, but was suppressed by oligomycin. We showed further that release of cytochrome c and the execution of apoptosis induced by ErPC3 can be inhibited by oligomycin. However, another inhibitor of this enzyme, piceatannol, inhibiting the water-exposed F1 component, did not affect ErPC3-mediated apoptosis. In addition, we analysed a possible correlation between ErPC3, MPTP, and H^+-ATP-synthase by investigating cellular ATP levels. ErPC3 reduced cellular ATP levels in U87MG and U118MG cells, while co-administration of ErPC3 with oligomycin or cyclosporin A restored cellular ATP levels.

Together, these results suggest a role of the oligomycin-sensitive F0 component of H^+-ATP-synthase in ErPC3-induced PTP opening and apoptosis.

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Chromogranin A-derived vasostatin-1 contains a sequence homologous to ezrin-radixin-moesin binding phosphoprotein 50 (EBP50) that regulates cell adhesion

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The circulating levels of chromogranin A (CgA), a protein stored in the secretory granules of many neuroendocrine cells, are increased in patients with various neuroendocrine tumors, with important diagnostic and prognostic implications. We have shown previously that production of CgA by neoplastic cells can affect tumor growth and morphogenesis by affecting the tumor microenvironment. In vitro studies showed that CgA and its N-terminal fragment CgA 1-78, called vasostatin-1 (VS-1) can modulate, in a negative or a positive manner respectively, fibroblasts and endothelial cell adhesion. In the present study we report a novel mechanism for the regulation of cell adhesion by recombinant VS-1. We used NIH-3T3 fibroblasts as a model to investigate its mechanism of action. We observed that these cells express a large number of non-saturable binding sites for the C-terminal region of VS-1 (residues 47-78). Furthermore, the C-terminal, but not the N-terminal, region of cell-bound VS-1 was resistant to degradation by proteinase K, indicating that VS-1 was bound to a protease-resistant structure through the C-terminal domain. This domain encompasses residues 47-66, an α-helix able to interact with membrane phospholipids, and residues 69-75 (AKERAHQ) sharing sequence similarity with ezrin-radixin-moesin (ERM) binding phosphoprotein 50 (EBP-50), a protein that by one hand binds members of the ERM family, by the other hand binds transmembrane proteins. Studies with recombinant CgA 1-78 and CgA 1-65 fragments showed that the C-terminal domain containing the AKERAHQ region is critical for cell adhesion. These results suggest that VS-1 regulates cell adhesion by interacting with cell membrane through the 47-66 residues and potentially with other membrane or cytoplasmic proteins with its C-terminal domain. Based on the high sequence homology with EBP50, interaction of the C-terminus with membrane or intracellular proteins involved in the regulation of membrane-cytoskeleton interactions could be a critical mechanism.

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Novel therapy for malignant pleural mesothelioma using 3-bromopyruvate based on anti-energetic effect

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Malignant mesothelioma is a poor prognosis cancer because it is resistant to chemotherapy and radiotherapy. Here, we tested a new anti-cancer approach based on the fact that cancer cells have an impaired metabolism of the glucose, leading to the secretion of lactic acid, a phenomenon first described by Otto Warburg, more than 80 years ago. We studied the effect of 3-Bromopyruvate (3-BrPA), first in vitro, on two human mesothelioma cell lines, either sensitive (MSTO-211H) or resistant (NCI-H28) to cisplatin, and then, in nude mice developing peritoneal carcinomatosis after intra peritoneal injection of MSTO-211H cells. 3-BrPA is an analogue of pyruvate that plays key role in the energetic metabolism. It has been considered to be able to inhibit hexokinase (HK), the first key enzyme of the glycolysis. HK, associated with phospho-Bad and VDACI, form a complex on the external mitochondrial membrane inhibiting the apoptosis. The removal of HK from this complex allows Bad dephosphorylation and thus apoptosis induction.

In both cell lines, 3-BrPA slowed down the proliferation without apoptosis induction. However, resistant NCI-H28 cells massively died in response to high 3-BrPA concentrations (up to 100μM), mainly by necrosis-poisoning mechanism. In contrast, when 3-BrPA was administered immediately when the cells are seeded in the flask (i.e. on detached cells), a massive apoptotic cell death was observed in MSTO-211H in response to low concentrations of 3-BrPA (50μM), involving the mitochondrial pathway, whereas no apoptotic cell death was observed in cisplatin-resistant NCI-H28.

In vivo, 3-BrPA increased the survival very significantly ($p<0.0001$), whereas cisplatin had no demonstrable effect. Using this novel anti-energetic agent, we think it is nowadays possible, either to slow down the proliferation and perhaps to facilitate action of other strategies, or to directly provoke cell death either by the apoptotic pathway or by necrosis-poisoning mechanisms. 3-BrPA could thus constitute and interesting novel anticancer drug which could be included in clinical trials, either to allow tumour regression or to impede tumour cells adhesion and subsequent peritoneal carcinomatosis or distant organ metastasis.

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Concomitant inhibition of Bcl-xL and Mcl-1 expression by RNA interference as a novel strategy for the treatment of ovarian carcinomas

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In ovarian cancers, cisplatin exposure of sensitive cells is associated with a down-regulation of Bcl-xL expression and apoptotic cell death whereas recurrence is systematically observed when Bcl-xL expression is maintained. We thus developed a specific siRNA targeting siXL1, and evaluated its ability to induce apoptotic cell death in response to cisplatin in resistant SKOV3 cells. siXL1 led to the disappearance of Bcl-xL mRNA and protein, associated with a low rate of apoptosis. In contrast, when siXL1 was combined to cisplatin, a massive cell death was observed whereas cisplatin alone was only transiently cytostatic. Thus, the inhibition of Bcl-xL expression could constitute a chemosensitizing way for the treatment of ovarian carcinoma.

Next, we investigated siXL1 effect on survival of intraperitoneal SKOV3 tumour bearing mice. The administration of siXL1 induced a strong increased of survival rate (median survival over 150 days in siXL1 group vs. 60-70 days in control groups). The surviving mice did not present any sign of residual tumour. Moreover, this effect on survival was associated with a significant decreased in both Bcl-xL expression and proliferation in tumour nodes, 5 days after siXL1 administration. In addition, a potential effect on angiogenesis is suspected but further investigations are now required to demonstrate its importance. However, a relative heterogeneity was noted in

response to siXL1 both at the molecular and survival levels suggesting that it remains possible to increase the effect of siXL1 by optimizing the delivery conditions.

Otherwise, considering that the concomitant inhibition of Bcl-xL and Mcl-1 expression could increase the efficiency of our strategy, as suggested in the literature, the interest of such combination was evaluated in vitro. We showed that neither siXL1 nor siMCL1 alone induced cell death whereas the combination of these siRNA induced a massive apoptosis. This observation shows that Bcl-xL and Mcl 1 appeared able to cooperate to protect ovarian carcinoma cells against apoptosis, either in response to oncogenic stress (providing a clinical advantage) or in response to chemotherapy. We are now analyzing whether the association of siXL1/siMCL1 with cisplatin could avoid the long term recurrence. Such multitargeted therapy could be also of interest for the treatment of ovarian carcinoma, in combination with conventional chemotherapy. These results are currently under in vivo preclinical validation.

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MCL-1 is an important determinant of the apoptotic response to the BH3-mimetic molecule HA14-1 in cisplatin resistant ovarian carcinoma cells

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Chemoresistance and in vitro recurrence of ovarian carcinoma have been previously associated to the absence of down-regulation of Bcl-xL expression in response to cisplatin. Our team is therefore developing various strategies to impede Bcl-x_L activity and/or expression, among which the use of BH3-mimetic molecules. These compounds reproduce the l structure of the BH3 domain of the Bcl-2 family members; they are able to induce apoptosis by dissociation of bax-like pro-apoptotic multidomain proteins from their anti-apoptotic partners.

We evaluated the interest of one of them, HA14-1, on various ovarian carcinoma cells lines resistant or sensitive to cisplatin. Differences were observed in response to HA14-1 in these cell lines, one of them undergoing strong apoptotic cell death (IGROV1-R10) whereas the others presented only a partial response (IGROV1, SKOV3, A2780) or an absence of response (OAW42). However, the sensitivity to HA14-1 was unrelated to the level of sensitivity to cisplatin, and the expression of HA14-1 targets (Bcl-2 and Bcl-x_L) was not correlated to these different responses.

In contrast, the lost of MCL-1 seemed associated with cell death in response to HA14-1, whereas maintenance or increase of MCL-1 expression led to resistance to this agent.

We therefore attempted about the importance of MCL-1 in the response to HA14-1 in SKOV3 and OAW42 resistant cells, and decided to inhibit its expression using a siRNA targeting MCL-1. Our results showed that siMCL-1 did not induced apoptosis on its own in these cells, whereas its association with HA14-1 induced a massive cell death. This results suggests that MCL-1 could cooperate with others Bcl-2 family members (e.g. Bcl-x_L) to protect ovarian carcinoma cells against oncogenic stress-induced apoptosis.

We also demonstrated that in SKOV3 cells (both resistant to cisplatin and to HA14-1), cisplatin was able to decrease MCL-1 expression, and that the association of HA14-1 with cisplatin induced a massive cell death, whereas cisplatin or HA14-1 alone was only transiently cytostatic.

These results suggest that MCL-1 is essential for the response to various apoptotic stimuli (oncogenic stress or conventional chemotherapy), and present resistant ovarian carcinomas as pertinent targets for the use of BH3-mimetics. Our work also showed that a siRNA directed against MCL-1 could interestingly reinforce the action of such molecules for the treatment of ovarian cancers refractory to conventional chemotherapy.

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Incorporation of a targeting ligand into adenovirus capsid as a mean of delivery of anti-angiogenic factors into tumors

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Inhibition of new vessels formation constitutes an approach to prevent tumor growth because angiogenesis is a mandatory step for tumor development. This can be achieved by delivery of genes able to modulate endothelial cells growth. Adenovirus (Ad) are potent tools to deliver genes because they can be produced at high titers and display high transduction efficiencies in a wide range of cell types. However, different studies indicated that gene transfer by Ad vectors into endothelial cells was confronted with poor transduction efficiency due to the lack of expression of the primary Ad receptor.

In order to improve gene transfer in endothelial cells, we inserted a targeting peptide (CNGRC motif) into different Ad capsid proteins. This peptide was previously shown to bind to aminopeptidase N (APN), a receptor expressed by neovessels. Compared to Ad bearing a wild-type capsid (Adwt), Ad bearing this peptide either into fiber protein (AdFNGR) or hexon protein (AdHNGR) were shown to achieve a higher β-galactosidase (β-gal) expression, in different endothelial cell lines (EAhy.926, SLK, CPAE) but also in human primary vascular endothelial cells. AdHNGR was also found to transduce more efficiently different tumor cells (LLC, RD and WEHI). We confirmed a role of APN in this new entry pathway since APN-specific uncompetititive (curcumin) and competitive (PC-18) inhibitors were able to reduce the ability of NGR-bearing Ad to transduce LLC cells. AdHNGR were also able to transduce some APN-negative cells (MDA-MB-435, L929) that are poorly transduced by Adwt. Binding assays studies showed that this property could be related to their ability to interact with αvβ3 integrins through NGR motifs.

Direct administration of AdHNGR into carcinoma (LLC) pre-established in nude mice led to a level of gene transfer comparable to Adwt. However, when AdHNGR was pseutotyped by an Ad3 fiber (targeting CD46, a receptor not expressed in mice), as a first approach of detargeting, we observed a 4-fold higher β-gal expression in LLC tumors compared to tumors injected with Adwt pseudotyped with an Ad3 fiber.

Altogether, our results pointed out that AdHNGR is very potent to transduce endothelial cells and emphasized that hexon protein could constitute a better alternative to fiber protein for incorporation of targeting ligands. Experiments are currently conducted to assess the ability of AdHNGR to deliver more efficiently angiostatin K1-5 into tumors and to decrease tumor growth.

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RNA interference-based strategies directed against Bcl-xL and MCL1 for the treatment of malignant pleural mesothelioma

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Malignant pleural mesothelioma (MPM) is a highly aggressive tumour with poor prognosis and limited response to standard combined chemotherapy (i.e. pemetrexed plus cisplatin). Anti-apoptotic proteins with altered expression have been previously described to contribute to chemoresistance, and among them, Bcl-xL seems to play an important role in MPM. Different strategies have been developed to impede its activity (BH3-mimetics) or its expression (antisense oligonucleotides). Interestingly, since it based on the highly specific and efficient silencing of a target gene, RNA interference (RNAi) represents one of the most promising innovating approaches to be combined to conventional therapies. In our study, a Bcl-xL specific RNA interference approach (siXL1) was used to inhibit Bcl-xL expression in mesothelioma cell lines for evaluating both its antitumor effect and its potential to sensitize mesothelioma cells to standard chemotherapy. We showed that siXL1 induced a drastic inhibition of Bcl-xL expression both at the mRNA and protein levels in different MPM cell lines. We characterized the response of chemoresistant NCI H28 cells to siXL1, alone or associated to cisplatin. siXL1 alone caused death of a fraction of the population (about 20%), the majority of cells being only transiently arrested in the cell cycle for few days. Notably, the combination of siXL1 and cisplatin resulted in a supra-additive effect with nearly complete annihilation of the population, whereas neither cisplatin alone nor cisplatin associated to control siRNA induced cell death in these cells. Although the observed cell death presented some features of apoptosis, its nature remains to be fully determined. Moreover, it was recently demonstrated that the neutralization of both Bcl-xL and MCL1 suffices for efficient Bak-mediated apoptosis. We thus evaluated the interest of the siXL1/siMCL1 combination and showed that this association is sufficient to induce a significant cell death. The interest of such siRNAs association combined to standard chemotherapy for the prevention of long term recurrence is under investigation. Finally, preclinical studies will be performed in nude mice to precise the therapeutic potential of such approaches for the treatment of MPM. In summary, these findings highlight that siRNA strategy aimed at down-regulating both Bcl-xL and MCL1 may be used as novel and highly effective tool, with the potential for future targeted therapy of malignant pleural mesothelioma.